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Multiple CTX-M-Type Extended-Spectrum β-Lactamases in Nosocomial Isolates of *Enterobacteriaceae* from a Hospital in Northern Italy

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Twelve isolates of *Enterobacteriaceae* (1 of *Klebsiella pneumoniae*, 8 of *Escherichia coli*, 1 of *Proteus mirabilis*, and 2 of *Proteus vulgaris*) classified as extended-spectrum β-lactamase (ESBL) producers according to the ESBL screen flow application of the BD-Phoenix automatic system and for which the cefotaxime MICs were higher than those of ceftazidime were collected between January 2001 and July 2002 at the Laboratory of Clinical Microbiology of the San Matteo University Hospital of Pavia (northern Italy). By PCR and sequencing, a CTX-M-type determinant was detected in six isolates, including three of *E. coli* (carrying $bla_{\text{CTX-M-1}}$), two of *P. vulgaris* (carrying $bla_{\text{CTX-M-2}}$), and one of *K. pneumoniae* (carrying $bla_{\text{CTX-M-15}}$). The three CTX-M-1-producing *E. coli* isolates were from different wards, and genotyping by pulsed-field gel electrophoresis (PFGE) revealed that they were clonally unrelated to each other. The two CTX-M-2-producing *P. vulgaris* isolates were from the same ward (although isolated several months apart), and PFGE analysis revealed probable clonal relatedness. The $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$ determinants were transferable to *E. coli* by conjugation, while conjugative transfer of the $bla_{\text{CTX-M-15}}$ determinant from *K. pneumoniae* was not detectable. Present findings indicate that CTX-M enzymes of various types are present also in Italy and underscore that different CTX-M determinants can be found in a single hospital and can show different dissemination patterns. This is also the first report of CTX-M-2 in *P. vulgaris*.

The CTX-M-type enzymes are a group of molecular class A extended-spectrum β-lactamases (ESBLs) that exhibit an overall preference for cefotaxime (CTX; hence the CTX-M name) and ceftriaxone and a higher susceptibility to tazobactam than to clavulanate. These enzymes are emerging in members of the family *Enterobacteriaceae*, where they can cause resistance to CTX and other expanded-spectrum β-lactams (7, 28).

Several different variants of CTX-M-type enzymes have been identified to date. They are clustered in at least six major evolutionary lineages (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and Toho-2), named after the enzyme first discovered for each lineage (5; amino acid sequences for TEM, SHV, and OXA extended-spectrum and inhibitor-resistant β-lactamases are found at http://www.lahey.org/studies/webt.stm). Members of different lineages differ at 10 to 30% of the amino acid residues, while most lineages include a number of minor variants that may differ from each other by one or a few amino acid substitutions (http://www.lahey.org/studies/webt.stm). Members of the CTX-M-2 and CTX-M-8 lineages are most likely derived from the mobilization of chromosomal β-lactamase genes of *Kluyvera ascorbata* and *Kluyvera georgiana*, respectively (12, 20), while the original sources of the other enzymes of this group remain unknown. Enzymes closely related to the CTX-M group include the chromosomal β-lactamases of *Klebsiella oxytoca*, *Proteus vulgaris*, *Citrobacter diversus*, *Serratia fonticola*, and *Kluyvera cryocrescens* (7, 9).

The $bla_{\text{CTX-M}}$ genes are often carried on transferable plasmids (28). Two of them ($bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-9}}$) were found to be associated with complex class 1 integrons related to In6 and In7, although they are not found on typical gene cassettes (1, 10, 23).

The CTX-M-type enzymes were first reported in South America (M. Radice, P. Power, J. Di Conza, and G. Gutkind, Letter, Antimicrob. Agents Chemother. **46:**602-603, 2002), Germany (4), and France (3). Subsequently reports showed that they are present in several European countries (2, 6, 7, 11, 24; I. Alobwede, F. H. M'Zali, D. M. Livermore, J. Heritage, N. Todd, and P. M. Hawkey, Letter, J. Antimicrob. Chemother. **51:**471-473, 2003), as well as in the Far East (7, 8, 29, 30), India (14), Russia (M. Pimkin, M. Edelstein, I. Palagin, A. Narezkina, and L. Stratchounski, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1874, 2002) and North America (E. S. Moland, S. Pottumarthy-Boddu, J. A. Black, A. Hossain, N. D. Hanson, and K. S. Thomson, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. LB-13, 2002).

In this work we report on the first detection of different CTX-M-type enzymes (CTX-M-1, CTX-M-2, and CTX-M-15) in clinical isolates of *Enterobacteriaceae*, including *Escherichia*

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coli, *P. vulgaris*, and *Klebsiella pneumoniae*, from an Italian hospital. We also report on the first detection of CTX-M-2 in *P. vulgaris*.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains analyzed in this study included 12 clinical isolates of *Enterobacteriaceae*, each from a different patient, that upon susceptibility testing with the NMIC/ID 4 panel of BD-Phoenix (BD Diagnostic Systems Europe, Le Pont de Claix, France) were classified as ESBL producers by the ESBL screen flow application of the same system. The CTX MICs for these isolates were found to be higher than those of ceftazidime (CAZ) in conventional microdilution susceptibility testing. The 12 isolates were from inpatients at the San Matteo IRCCS Hospital of Pavia (northern Italy) during the period January 2001 to July 2002 and were identified with the GNID panel of BD-Phoenix. For *P. vulgaris* isolates, the identification was confirmed by the GNI card of the Vitek system (BioMérieux, Rome, Italy) and by the API 20E identification system (BioMérieux).

Susceptibility testing. MICs of CTX, CAZ, cefepime (FEP), and aztreonam (ATM) were determined by a microdilution test using cation-adjusted Mueller-Hinton (MH) broth (Difco Laboratories, Detroit, Mich.), in accordance with the criteria of the National Committee for Clinical Laboratory Standards (15). The MIC panels were prepared in house. Plates were incubated at 35°C for 18 h before recording results. *E. coli* ATCC 25922 was used as a reference strain for quality control of in vitro susceptibility testing. Susceptibilities to other antimicrobial agents were reported according to data from the BD-Phoenix system.

β-Lactamase assays. ESBL production was initially screened for by the ESBL screen flow application of BD-Phoenix. ESBL production was confirmed by a double-disk test to screen for synergy between serine β-lactamase inhibitors (clavulanate and tazobactam) and oxyimino cephalosporins or ATM (13). Commercial disks of amoxicillin-clavulanate and piperacillin-tazobactam (BD Diagnostic Systems) were used as sources of inhibitors. Inhibitor-containing disks were placed 22.5 mm apart (center to center) from those containing the oxyimino cephalosporins and ATM. Analytical isoelectric focusing (IEF) of crude extracts, detection of β-lactamase bands by nitrocefin, and detection of the activity of the β-lactamase bands separated by IEF against β-lactam substrates by a substrate overlaying procedure were assayed as reported previously (18), with an antibiotic concentration of 1 μg/ml in the medium overlay and with *E. coli* ATCC 25922 as an indicator strain. Substrate hydrolysis was revealed by the occurrence of bacterial growth above the enzyme bands. Reference strains producing TEM-1, TEM-2, TEM-7, TEM-8, TEM-9, TEM-12, SHV-1, SHV-2, SHV-5, and MIR-1 were used as controls, as described previously (18).

Molecular analysis techniques. PCR amplification of $bla_{\text{CTX-M}}$ alleles was carried out with primers CTX-MU1 (5′-ATGTGCAGYACCAGTAARGT) and CTX-MU2 (5′-TGGGTRAARTARGTSACCAGA), designed on conserved regions of $bla_{\text{CTX-M}}$ genes, including $bla_{\text{CTX-M-1}}$ to $bla_{\text{CTX-M-30}}$, $bla_{\text{TOHO-1}}$ to $bla_{\text{TOHO-3}}$, $bla_{\text{FEC-1}}$, $bla_{\text{UOE-1}}$, and $bla_{\text{UOE-2}}$ (http://www.lahey.org/studies/webt.stm; EMBL/GenBank accession numbers AB059404, AB098539, AF311345, AY156923, and AY238472). These primers target amplification of a 593-bp internal region of the $bla_{\text{CTX-M}}$ genes. The following reaction parameters were used: initial denaturation at 94°C for 7 min; denaturation at 94°C for 50 s, annealing at 50°C for 40 s, and elongation at 72°C for 60 s, repeated for 35 cycles; final extension at 72°C for 5 min. PCR amplification of the allele belonging in the $bla_{\text{CTX-M-3/15/22}}$ group was carried out with primers CTX-M3G-F (5′-GTTACA ATGTGTGAGAAGCAG) and CTX-M3G-R (5′-CCGTTTCCGCTATTACA AAC) and the following reaction parameters: initial denaturation at 94°C for 7 min; denaturation at 94°C for 50 s, annealing at 50°C for 40 s, and elongation at 68°C for 60 s, repeated for 35 cycles; final extension at 68°C for 5 min. PCR was always carried out in a 50-μl volume with 30 pmol of each primer, 200 μM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, and 0.5 U of the Expand PCR system (Roche Biochemicals, Mannheim, Germany) in the reaction buffer provided by the enzyme manufacturer. Direct sequencing of PCR products was carried out as described previously (22) with custom sequencing primers. Both strands were sequenced. Plasmid DNA was extracted by the alkaline lysis method (25). Colony blot hybridization was carried out as described previously (25); final washing was performed with 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) (or 2× SSC for low-stringency conditions) at 65°C. Southern hybridization was carried out directly on dried gels as described previously (27) with DNA probes labeled with ^{32}P by the random-priming technique. The $bla_{\text{CTX-M}}$ probe was made by a 1:1 mixture of amplicons generated with the CTX-MU1 and CTX-MU2 primers from $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$, respectively.

Conjugation assays. Conjugal transfer of resistance determinants was assayed in liquid medium (21) with the *E. coli* K-12 strain J62 (*pro his trp lac* Sm^r) as the recipient. Donor strains in the logarithmic phase of growth were mixed with recipients in early stationary phase in a 1:10 ratio in MH broth, and the mixture was incubated at 37°C for 14 h. Transconjugants were selected on MH agar containing CTX (2 μg/ml) plus streptomycin (1,000 μg/ml). The detection sensitivity of the assay was approximately 10^{-8} transconjugants per recipient.

PFGE. Pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA were analyzed by means of the Gene Path procedure (Bio-Rad Laboratories, Richmond, Calif.) using the no. 5 pathogen group reagent kit and the restriction enzyme *Sfi*I for *P. vulgaris* and the no. 2 pathogen group reagent kit and the restriction enzyme *Not*I for *E. coli*. DNA fragments were electrophoresed in 1% agarose gels in 0.5× Tris-borate-EDTA buffer with the Gene Path system (Bio-Rad) at 14°C and 6 V/cm for 20 h, with pulse times ranging from 5 to 50 s. Bacteriophage λ concatemers (Bio-Rad) were used as DNA size markers. Clonal relationships based on PFGE patterns were interpreted according to the criteria proposed by Tenover et al. (26).

RESULTS AND DISCUSSION

Detection of CTX-M-producing isolates. During the period January 2001 to July 2002, at the Laboratory of Clinical Microbiology of the IRCCS San Matteo Hospital of Pavia, a total of 2,682 samples positive for enteric bacteria were processed and 232 ESBL producers were identified by the ESBL screen flow application of the BD-Phoenix system. Twelve of the putative ESBL producers, for which the CTX MICs were higher than those of CAZ in a conventional microdilution susceptibility test, were investigated in this study. The isolates included eight of *E. coli*, two of *P. vulgaris*, one of *Proteus mirabilis*, and one of *K. pneumoniae* (Table 1).

MICs of extended-spectrum cephalosporins and ATM exhibited a notable variability among different isolates, but they were always ≥2 μg/ml, except for the 1-μg/ml MIC of FEP observed with the *E. coli* isolate EC26SM02 (Table 1). All isolates were resistant to ampicillin, amoxicillin-clavulanate, piperacillin, and piperacillin-tazobactam, and most isolates were also resistant to ciprofloxacin, levofloxacin, and gentamicin (Table 1). All isolates were susceptible to carbapenems (imipenem and meropenem) and to amikacin, except *P. vulgaris* PV19SM02, which was intermediate to the latter drug.

A double-disk synergy test yielded a positive result in all cases, although with variable detection sensitivity with different substrate-inhibitor combinations. In particular, clavulanate synergy with either oxyimino cephalosporins or ATM was not detectable for one *P. vulgaris* isolate (PV19SM02) and one *E. coli* isolate (EC26SM02); with the same *E. coli* isolate tazobactam synergy was detectable only with FEP (Table 1).

The resistance phenotypes of the 12 isolates were suggestive of production of a CTX-M-type ESBL. Screening for $bla_{\text{CTX-M}}$ determinants by PCR with the CTX-MU1 and CTX-MU2 primers, designed on conserved regions of $bla_{\text{CTX-M}}$ genes, yielded an amplification product of the expected size (0.6 kb) from six isolates, including three of *E. coli*, two of *P. vulgaris*, and one of *K. pneumoniae* (Table 1). Sequencing the amplification products from the three *E. coli* isolates identified the resistance gene as $bla_{\text{CTX-M-1}}$ (Table 1). The genes in the two *P. vulgaris* isolates were identified as $bla_{\text{CTX-M-2}}$ (Table 1). The gene in the *K. pneumoniae* isolate was identified as $bla_{\text{UOE-1}}$, $bla_{\text{CTX-M-15}}$, or $bla_{\text{CTX-M-28}}$. In this case, amplification of the entire coding sequence with primers CTX-M3G-F and CTX-M3G-R, followed by direct sequencing, identified the resistance gene as $bla_{\text{CTX-M-15}}$ (Table 1). Colony blot hybridization

TABLE 1. Antimicrobial susceptibility, β-lactamase production, and bla_{CTX-M} genes in the isolates of *Enterobacteriaceae* analyzed in this study

Species	Isolate	MIC (μg/ml) of:				Result of DD synergy test[a] with:		pI(s) by IEF (substrate[s] hydrolyzed)[b]	bla_{CTX-M} gene	Other drugs to which resistance was shown[c]
		CTX	CAZ	FEP	ATM	CLA	TAZ			
K. pneumoniae	KPISM01	>128	2	>128	>128	+	+	8.9 (CTX, FEP, ATM), 7.6, 5.4	$bla_{CTX-M-15}$	AM, AC, PI, PT, GM
E. coli	EC10SM01	64	16	16	32	+	+	8.6 (CTX, FEP, ATM), 5.4	$bla_{CTX-M-1}$	AM, AC, PI, PT, CI, LE, GM
	EC14SM02	128	8	64	32	+	+	8.6 (CTX, FEP, ATM), 6.5	$bla_{CTX-M-1}$	AM, AC, PI, PT, CI, LE
	EC21SM02	>128	2	8	64	+	+	8.6 (CTX, FEP, ATM), 5.4	$bla_{CTX-M-1}$	AM, AC, PI, PT, CI, LE
	EC11SM02	8	4	8	16	+	+	8.4 (CTX, FEP, ATM), 6.5, 5.4	—[d]	AM, AC, PI, PT, CI, LE, GM
	EC17SM02	64	32	4	16	+	+	8.0 (CTX, FEP, ATM), 5.4	—	AM, AC, PI, PT, CI, LE, GM
	EC26SM02	32	16	1	16	−	+ (FEP only)	>9.0 (CTX, CAZ, FEP), 5.4	—	AM, AC, PI, PT, CI, LE, GM
	EC27SM02	>126	16	2	64	+	+	8.0 (CTX, FEP, ATM), 5.4	—	AM, AC, PI, PT, CI, LE, GM
	EC28SM01	>128	16	2	64	+	+	8.0 (CTX, FEP, ATM), 5.4	—	AM, AC, PI, PT, CI, LE, GM
P. vulgaris	PV1SM01	32	16	2	32	+	+	8.0 (CTX, FEP, ATM), 7.6, 5.4	$bla_{CTX-M-2}$	AM, AC, PI, PT, CI, LE, GM
	PV19SM02	>128	16	8	>128	−	+	8.0 (CTX, FEP, ATM), 7.6 (CTX); 5.4	$bla_{CTX-M-2}$	AM, AC, PI, PT, CI, LE, GM
P. mirabilis	PM15SM02	32	4	8	32	+	+	8.4 (CTX, FEP, ATM) 5.9 (CTX, CAZ, FEP, ATM), 5.4	—	AM, AC, PI, PT, CI, LE, GM

[a] CLA, clavulanate; TAZ, tazobactam; +, detectable synergy between the β-lactamase inhibitor and CTX, CAZ, FEP, and ATM in the double-disk (DD) test; −, absence of detectable synergy between the β-lactamase inhibitor and the extended-spectrum β-lactams.
[b] The pIs of the β-lactamase bands are indicated; the extended-spectrum β-lactams hydrolyzed by each band are in parentheses.
[c] AM, ampicillin; AC, amoxicillin-clavulanate; PI, piperacillin; PT, piperacillin-tazobactam; CI, ciprofloxacin; LE, levofloxacin; GM, gentamicin.
[d] —, no bla_{CTX-M} gene detected.

analysis of the 12 isolates using a $bla_{CTX-M-1/2}$ probe mixture yielded results that were fully consistent with those of PCR screening (data not shown). The PCR-negative isolates were not recognized by the probe mixture, even under low-stringency hybridization conditions, suggesting that ESBLs other than those of the CTX-M type were produced by these isolates.

Analytical IEF of crude extracts of the 12 isolates revealed heterogeneous patterns, with multiple β-lactamase bands in all cases (Table 1). Some of these bands exhibited activity against oxyimino cephalosporins and ATM in a bioassay. The nature of CTX-M-type enzymes detected by molecular analysis was consistent with the presence of a pI 8.9 ESBL active on CTX, FEP, and ATM in the *K. pneumoniae* isolate, of a pI 8.6 ESBL active on the same substrates in the three CTX-M-positive *E. coli* isolates, and of a pI 8.0 ESBL active on the same substrates in the two *P. vulgaris* isolates. Although CTX-M-15 has been reported to be active also on CAZ (19), activity against this substrate was not detectable in the bioassay, probably because of the relatively low catalytic efficiency exhibited by CTX-M-15 against this substrate (19). The natures of the ESBLs in the CTX-M-negative isolates and of the other enzymes were not investigated in this work and will be the subject of another investigation.

Clonal relationships and distribution of the CTX-M-producing isolates. The PFGE profiles of genomic DNA of the *P. vulgaris* isolates producing CTX-M-2, digested with *Sfi*I, differed from each other by only two bands (Fig. 1A), revealing probable clonal relatedness. On the other hand, the PFGE profiles of genomic DNA of the three *E. coli* isolates producing CTX-M-1, digested with *Not*I, were notably different (by more than four bands), indicating that these isolates were clonally unrelated (Fig. 1B).

The six CTX-M-producing isolates exhibited a different clinical distribution. The *K. pneumoniae* isolate producing CTX-M-15 was an apparently sporadic isolate from the Hematology ward. The two *P. vulgaris* isolates producing CTX-M-2 were isolated from the same long-term care ward, although several months apart. The three *E. coli* isolates producing CTX-M-1 were each from a different ward (Table 2).

Transferability of the bla_{CTX-M} genes. Transferability of the CTX-M determinants was assayed in mating experiments using an *E. coli* recipient and CTX for selection of transconjugants. Results of these experiments showed that the CTX-M-1 determinant was transferable from each *E. coli* donor at a frequency of approximately 10^{-4} transconjugants/recipient, while the

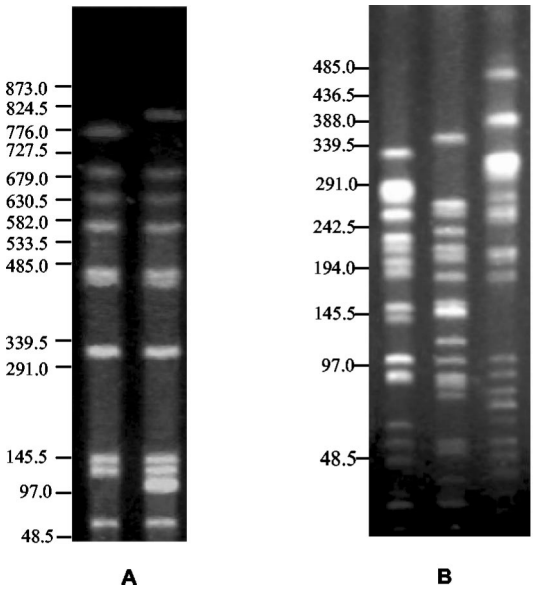

FIG. 1. (A) PFGE profiles of genomic DNAs of the CTX-M-2-producing *P. vulgaris* isolates digested with *Sfi*I. Left lane, isolate PV1SM01; right lane, PV19 isolate PV19SM02. (B) PFGE profiles of genomic DNAs of the CTX-M-1-producing *E. coli* isolates digested with *Not*I. Left lane, isolate EC14SM02; middle lane, isolate EC21SM02; right lane, isolate EC10SM01. DNA size standards (in kilobases) are indicated on the left.

TABLE 2. Distribution, clinical features, and clonal relationships of CTX-M-producing clinical isolates of *Enterobacteriaceae*

Species	Isolate (enzyme)	Date	Ward	Specimen	PFGE profile
K. pneumoniae	KP1SM01 (CTX-M-15)	January 2001	Hematology	Blood	ND[a]
E. coli	EC10SM01 (CTX-M-1)	November 2001	Internal Medicine I	Urine	Unique
	EC14SM02 (CTX-M-1)	February 2002	Vascular Surgery	Gangrenous tissue	Unique
	EC21SM02 (CTX-M-1)	March 2002	Internal Medicine II	Urine	Unique
P. vulgaris	PV01SM01 (CTX-M-2)	January 2001	Long-Term Care I	Urine	A1
	PV19SM02 (CTX-M-2)	May 2002	Long-Term Care I	Decubitus ulcer	A2

[a] ND, not determined.

CTX-M-2 determinant was transferable from each *P. vulgaris* donor at a frequency of approximately 10^{-5} transconjugants/recipient. Conjugal transfer of the CTX-M-15 determinant carried by the *K. pneumoniae* isolate was not detectable. All *E. coli* transconjugants were recognized by the $bla_{\text{CTX-M-1/2}}$ probe mixture in a colony blot hybridization (data not shown), confirming that transfer of the CTX-M determinant had occurred.

Transconjugants derived from the CTX-M-1-positive *E. coli* strains produced a single pI 8.6 β-lactamase with ESBL activity and showed similar patterns of decreased susceptibility to oxyimino cephalosporins and ATM (Table 3). All these transconjugants apparently harbored the same plasmid, named pSMEC10, whose size was estimated to be approximately 50 kb according to results of restriction analysis (Fig. 2A) and which was recognized by the $bla_{\text{CTX-M-1/2}}$ probe mixture in a Southern hybridization experiment (data not shown). Transconjugants derived from the CTX-M-2-positive *P. vulgaris* isolates produced two β-lactamases, including a pI 8 ESBL and a pI 5.4 non-ESBL (that was likely TEM-1), and showed similar patterns of decreased susceptibility to oxyimino cephalosporins and ATM (Table 3). All these transconjugants apparently harbored the same plasmid, named pSMPV1, whose size was estimated to be approximately 55 kb according to results of restriction analysis (Fig. 2B) and which was recognized by the $bla_{\text{CTX-M-1/2}}$ probe mixture in a Southern hybridization experiment (data not shown). After digestion with the same enzyme (*Pst*I), plasmids pSMEC10 and pSMPV1 exhibited different restriction profiles (Fig. 2).

Concluding remarks. To our best knowledge this is the first report of the isolation of multiple CTX-M-type enzymes from an Italian hospital. Three enzyme classes, namely, CTX-M-1, CTX-M-2, and CTX-M-15, were detected in the same hospital. The three enzymes exhibited different distributions: CTX-M-1 was detected in *E. coli*, CTX-M-2 was detected in *P. vulgaris* (a species that was not previously reported among CTX-M producers), and CTX-M-15 was detected in *K. pneumoniae*. CTX-M-15 belongs in the CTX-M-1 lineage and has previously been detected in India, Japan, Bulgaria, and Poland (19). Compared to CTX-M-3, from which it differs by a single amino acid residue, it exhibits an increased activity on CAZ (19).

The different distributions of the three CTX-M determinants likely reflect differences in their genetic vehicles. Conjugational transfer could not be detected for the CTX-M-15 determinant carried by the *K. pneumoniae* isolate, while both the CTX-M-1 determinant found in *E. coli* and the CTX-M-2 determinant found in *P. vulgaris* were readily transferable by conjugation, although at different frequencies. Finding the same CTX-M-1-encoding plasmid in three clonally unrelated strains of *E. coli* from three different wards indicates a notable spreading potential for this plasmid and suggests that horizontal transfer could be the principal mechanism of spreading $bla_{\text{CTX-M-1}}$ in the hospital environment. On the other hand, the two *P. vulgaris* isolates producing CTX-M-2 appeared to be related to each other and were from the same ward, suggesting a clonal spread within that ward. The strains producing different enzymes were detected in different wards.

Isolates of *Enterobacteriaceae* producing ESBLs other than those of the CTX-M type had already been detected in the same hospital (16–18). The number of CTX-M-producing isolates was low, overall, considering the total number of enteric bacterial isolates and also the total number of ESBL producers isolated during the same period. However, note that the screening criterion adopted was based on simple phenotypic features

TABLE 3. Antimicrobial susceptibilities and β-lactamase production of CTX-M-positive *E. coli* transconjugants obtained in mating experiments[c]

Transconjugant[a] or strain (en By me)	MIC (μg/ml) of:				pI(s) by IEF (substrate hydrolyzed)
	CTX	CAZ	FEP	ATM	
J62 × EC10SM01 (CTX-M-1)	16	8	16	16	8.6 (CTX, FEP, ATM)
J62 × EC14SM02 (CTX-M-1)	32	4	16	16	8.6 (CTX, FEP, ATM)
J62 × EC21SM02 (CTX-M-1)	32	4	32	16	8.6 (CTX, FEP, ATM)
J62 × PV01SM01 (CTX-M-2)	32	2	16	2	8.0 (CTX, FEP, ATM), 5.4
J62 × PV19SM02 (CTX-M-2)	16	1	16	4	8.0 (CTX, FEP, ATM), 5.4
J62	0.03	0.25	0.06	0.25	ND[b]

[a] From each conjugation experiment, one transconjugant was randomly selected and subjected to in vitro susceptibility testing and IEF analysis.
[b] ND, no band of enzyme activity was detectable.
[c] Susceptibility of the J62 recipient strain is shown for comparison.

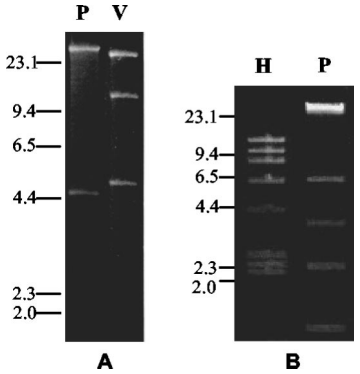

FIG. 2. (A) *Eco*RV and *Pst*I restriction profiles (lanes V and P, respectively) of the plasmid extracted from a transconjugant obtained with *E. coli* EC10SM01 as the donor. Plasmids from transconjugants obtained from *E. coli* EC14SM02 and EC21SM02 exhibited apparently identical restriction profiles and are not shown. (B) *Hin*dIII and *Pst*I restriction profile (lanes H and P, respectively) of the plasmid extracted from a transconjugant obtained using *P. vulgaris* PV1SM01 as the donor. A plasmid from a transconjugant obtained from *P. vulgaris* PV19SM02 exhibited an apparently identical restriction profile and is not shown. DNA size standards (in kilobases) are indicated on the left.

and that the number of CTX-M-producing isolates detected in this work could be underestimated. In fact, strains containing $bla_{\text{CTX-M}}$ genes but also producing additional enzymes resulting in CAZ MICs higher than those of CTX would have been missed. Systematic screening by molecular methods will be necessary to determine the prevalence of CTX-M-producing strains within our hospital. PCR screening using the CTX-MU primers, designed on highly conserved sequences of $bla_{\text{CTX-M}}$ genes, is expected to be most useful for this purpose.

Concerning phenotypic detection, the ESBL screen flow application of the BD-Phoenix system appeared to be capable of detection of strains producing ESBLs of the CTX-M type. When a double-disk diffusion test was used, tazobactam appeared to be slightly more sensitive than clavulanate for detection of similar ESBL producers.

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